Anopheles larval food and Pan Density

We receive numerous inquiries about anopheline larval food. Following are some experiments and recommendations. Although these experiments are with anophelines, those rearing Aedes and Culex should also have good results using this methodology due to their relatively robust biology.

Embryo to 2nd instar larvae

- Many foods appear suitable until one measures mortality from hatching to pupa. Our current recommendation for larval food is based on experiments measuring survival and growth rate. We recommend 2% w/v baker's yeast for the day of hatching and the 3rd day. (We recommend placing the 2% w/v baker's yeast into a 50 ml Falcon tube and filling to volume with distilled water.) Thereafter, switch to regular powder larval food.
- For vials containing iso-female progeny, feed two or three drops on the day that the larvae hatch (See Schedule). Larvae should be transferred to larger containers on the next day. When the larvae are transferred to a larger container, feed approximately the following volumes of food;
    - o <15 in an 8 oz. Cup, feed 0.1ml in 100 ml water
    - o 15-50 in small plastic boxes, feed 0.5 ml in 200 ml water
    - o 50-100 in a large plastic box, feed 1.0 ml in 300 ml water
    - o >100 in a large tray, feed 2.0 ml in 500-700 ml water

  It is important to not re-use the baker's yeast slurry. We have found that even refrigerated leftover slurry has caused an increase in bacterial/fungal growth in the rearing pans.

2nd to 4th instar larvae

Our long-time favorite, TetraMin Baby-E fish food, was discontinued as of 2001, so we conducted experiments in September of 2002 with 8 substitute foods designed for tropical fish. Fortunately, several of these were at least as good as Baby-E.

Dec 20, 2005 Latest larva food update

As a follow-up to the controlled experiments described below, we attempted to conduct experiments with a blend of one of the foods used in those experiments; Aquaricare Koi Floating Blend. While this food is still sold by Aquaricare, the recipe has been changed; the older recipe contained only green pellets, whereas the new one contains both green and reddish pellets. We have used the new formulation successfully for several years with the following species: *A. albimanus, A. atroparvus, A. farauti, A. freeborni, A. quadrimaculatus, A. gambiae, A. arabiensis, A. stephensi, A. minimus* and *Aedes aegypti*. Eggs are hatched into baker's yeast in water as described below. On the third day, larvae are split and thinned according to needs, and given 2 ml of baker's yeast and surface fed a pinch of finely ground Koi Floating Blend. We prepare this by grinding the Koi pellets in a grinding mill and sifting through a 250 micron mesh. On the fifth day, we thin larvae again and feed additional ground food daily until pupation.

Materials & Methods

The following foods were chosen for testing based on variety and advertised distinctiveness plus a bit of pure subjectivity:

- VitaPro Plus Cichlid Power Flakes
- AquariCare Tropical Floaters
- VitaPro Plus Staple Power Flakes
- Premi-Yum Brine Shrimp Flakes

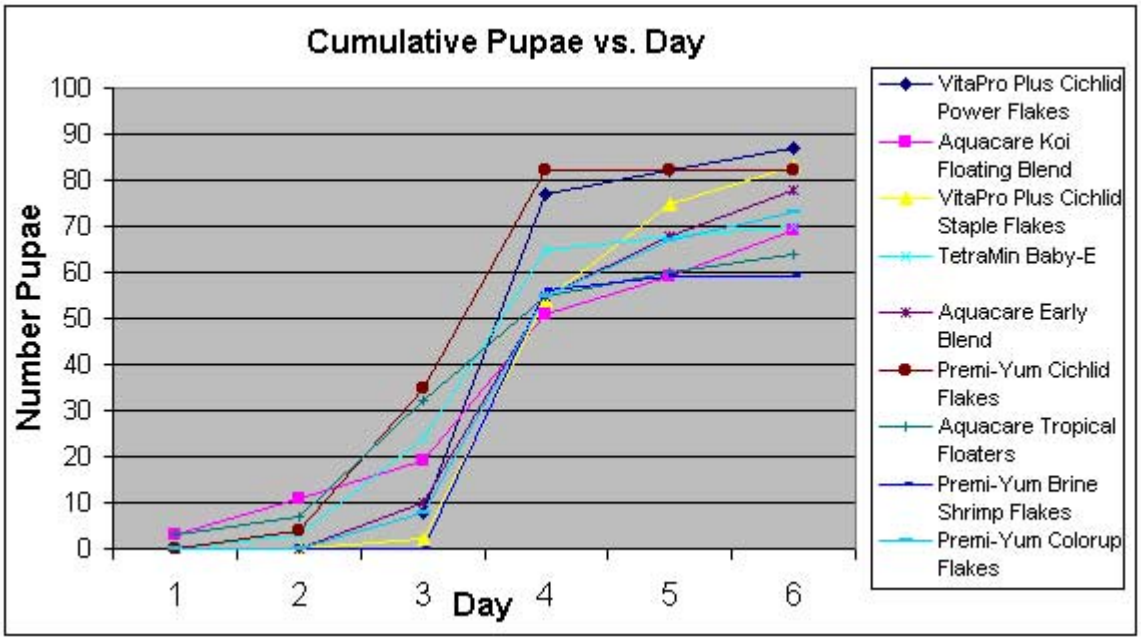
- Aquaricare Early Blend
- Aquaricare Koi Floating Blend
- Premi-Yum Colorup Flakes
- Premi-Yum Cichlid Flakes
- TetraMin Baby-E (discontinued)

Feeding Experiment

It is impossible to determine the quality of a food unless survival from initiation to pupation or adult emergence is known. It is not uncommon to experience extreme mortality among early stage larvae, yet the quality and number of L4s, pupae, and adults is acceptable. Therefore in the following experiments, we counted 100 L1s/L2s (two days after hatch) to begin the experiments and counted the number of pupae that formed each day. We assumed that egg laying would be adequate if larval development was rapid with little mortality. Three sets of experiments were performed with *A. gambiae, stephensi,* and *quadrimaculatus* GOCUT.
Feeding schedule used for food comparison:

- Day 1 Hatch in approx. 300 ml water with 2 ml 2% w/v active baker's yeast in water
- Day 2 No food or water added
- Day 3 Count 100 larvae into cups containing approx. 30 ml water
- Day 4 No food or water added
- Day 5 Add 0.25 ml food and 30 ml water
- Day 6 No food or water added
- Day 7 Add 1 ml food and 50 ml water
- Hereafter feed as needed. (We attempted to feed the same volume of all foods each day. Toward the end of each experiment, it was necessary to vary the food among groups according to estimated demand.)

Culture temperature was 27°C. A graph of one replicate using *Anopheles gambiae* is shown below.



Recommendations

The following recommendations are based on survival, pupation rate, and ease of use. The latter refers to our very subjective ability to see larvae and pupae against the background of feces and food debris and degree of water clouding. Highest weight was given to foods with which the GOCUT strain of *A.*

quadrimaculatus cultured well, because it is our stock most sensitive to poor culture conditions and is therefore the "canary" of our *Anopheles. A. stephensi* is least sensitive to poor culture with *albimanus* and *gambiae* in between. The following recommendations are quite good for those species. Having said that, all of the following food could be used for anopheline culture with some degree of success. No significant failure of adult emergence or differences in adult longevity were observed with any food. (Although AquariCare Koi Floating Blend fell into the "acceptable" group, we very much liked the clean green water and healthy looking larvae. This food might be good blended with one of the "top choices" for a very complete mix.)
Top choices: VitaPro Plus Cichlid Power Flakes and AquariCare Tropical Floaters. **Acceptable choices:** VitaPro-Plus Staple Power Flakes, Premi-Yum Brine Shrimp Flakes, AquariCare Early Blend, AquariCare Koi Floating Blend **Lowest choices**: Both Premi-Yum Colorup Flakes, Premi-Yum Cichlid Flakes clouded the water without significant growth rate enhancement above the others.

Vita-Pro and Premi-Yum products are available from www.mreed.com

Aquaricare products are available from www.aquaricare.org

Pan Density

There are several factors that can be controlled in the insectary that have been shown to affect mortality in anopheline mosquitoes: temperature, water depth, feeding, and larval density. It has been shown experimentally that eclosion rates diminish as density in the pan increases [1]. High larval density was also found to be a distorting factor in sex ratios that favored more males than females in *Anopheles stephensi* [2]. Larval conditions are therefore very important in colony maintenance.

1. Timmerman, SE and H Briegel. (1993) Water depth and larval density affect development and accumulation of reserves in laboratory populations of mosquitoes. **Bull. Soc. Vector Ecol**. 18(2) 174-187.
2. Reisen, WK and RW Emory. (1977) The effects of larval intraspecific competition on imaginal densities in *Anopheles stephensi* (Diptera: Culicidae): a laboratory evaluation. **Can. Ent**. 109 1481-1484.

The MR4 currently keeps their pans at a density of 200-300 larvae per pan (depending on the species). This number does not adversely affect the overall size of adults and allows for a large number of adults to be produced with only a few pans. Care must be taken to ensure water conditions are kept favorable due to the larger amount of waste created by more larvae.

As stated above, over-crowding larvae will lead to either smaller, less vigorous adults or fewer adults emerging. Although over-crowding is okay during the 1st and 2nd instars, it should be avoided thereafter, otherwise the colony will be stressed.

Although this is a very good way to have all your pans, it will change the rearing schedule. Larvae that are too thin will inevitably “speed up” their development rate and be unpredictable in when they will pupate. It is also difficult to adjust the amount fed so over or under feeding is more likely.